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Fatty Acids and Triglycerides Composition in Uruguayan Cow, Sheep and Goat Cheeses

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Abstract: Adverse health consequences are associated with consumption of so-called *trans* fatty acids (FAs) generated during chemical hydrogenation processes. Naturally-occurring *trans* fats, including *trans* vaccenic acid (TVA) derived from a conjugated isomer of linoleic acid (18:2 *cis*-9, *trans*-11), (CLA) in contrast, are recognized as having potentially beneficial effects on human health. These natural *trans* FAs are found in fat from dairy products and meat of ruminants, and have distinct effects compared with *trans* FAs industrially produced. The lipid composition of cheeses depends on the milk used. In this work was determined CLA and TVA content in milk fat of sheep and cow to be 1.4% and 3.2%, respectively, and from goat, 0.7% and 1.8%. Also, differences in CLA and TVA content were found in fat extracted from samples of the same cheese type made from cow's milk from the same company but with different production dates during a year. The levels of CLA and TVA found in Uruguayan cheeses were generally higher than levels reported in previous studies with comparable cheeses produced in other countries. Milk fat is well-known to confer specific properties to foods. This in turn will affect the rheology and sensory attributes as food. For this reason, it is of interest to determine the triglyceride (TAG) composition in milk-derived foods. Results show that there are characteristic differences in the TAG composition of the different cheeses. We found minimal differences between cheeses from goat and sheep but found distinct characteristics for TAGs from cow milk-derived cheese.

Key words: *Trans* fatty acids, vaccenic acid, conjugated linoleic acid, triglycerides, cheeses.

1. Introduction

Milk fat composition is highly variable in different mammalian species. Some fatty acids (FAs) found in milk fat of ruminants are absent in other terrestrial or marine mammals. In ruminants, occur changes in the composition of the FAs from the diet that in mammalian not happen because they incorporated practically the fatty acids of the intake without further modification. For example, there are large differences in content of short and medium chain length FAs (C4 to C14) as well as that of polyunsaturated FAs and *trans* isomers.

Triglycerides (TAGs) are the major constituents in milk fat accounting for 97%-98% of total lipid. The remaining components include phospholipids (0.8%) as well as sterols, free FAs, carotenoids and fat-soluble vitamins together accounting for less than 0.5% [1-4]. Walstra and Jenness [3] described the composition of TAGs in milk fat of ruminants as very complex in that an estimated 1,300 different TAGs were predicted considering only those containing FAs present at levels greater than 1%.

An extensive literature [5-13] indicated that fat composition in cow, sheep and goat milk is very different, as is the lipid composition of cheeses produced from these different milk sources.

The American Heart Association, in agreement with the FAO and WHO, proposed reducing consumption of industrially-generated *trans* FAs with the hope of

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helping to avoid cardiovascular disease. Nutrition research developed after 1990 [14-21] indicated a relatively negative effect on blood cholesterol transport by *trans* FAs compared with palmitic acid (16:0) and was associated with increased blood levels of TAGs- and LDL-cholesterol while lowering HDL-cholesterol. However, it is well-established that some *trans* FAs are generated from dietary lipids by biohydrogenation under "natural" conditions in the rumen. This process is responsible for the occurrence of up to 18 isomers of conjugated linoleic acids (CLA).

CLA comprises a family of positional and geometric isomers, all conjugated dienes of linoleic acid (18:2 *cis*-9, *cis*-12). The *cis*-9, *trans*-11 isomer (termed 9/11CLA) is the most abundant in dairy products (90% of total). CLA were found to have beneficial effects for health in that they are hypocholesterolemic and anticarcinogenic, and have antioxidant properties, enhance the immune system and help in weight reduction [22].

CLA are thus formed as a result of incomplete rumen biohydrogenation, and can therefore be considered natural constituents of milk products and fat from ruminants.

Biohydrogenation of unsaturated acids by rumen bacteria first results in isomerization of *cis*-12 linoleic acid to form the *cis*-9, *trans*-11 isomer (to form 9/11CLA). The second reaction is reduction of the *cis*-9 CLA double bond, and the FA becomes mono-unsaturated with 18 carbons. The structure formed with a single unsaturation, *trans*-11, is vaccenic acid, also termed *trans*-vaccenic acid (TVA). Reduction of the *cis*-9 double bond is faster than the preceding step resulting in accumulation of TVA in the rumen. There is another pathway for formation of 9/11CLA. This reaction takes place in the liver and mammary gland of ruminants. TVA produced in the rumen can be desaturated at carbon 9 (forming 9/11CLA) by the enzyme Δ9-desaturase. Activity is found in intestinal, hepatic and the mammary gland, and can convert TVA to CLA. Accordingly, the total content of 9/11CLA in ruminant milk is due to the sum of these two metabolic pathways [23-24].

Human Δ9-desaturase enzyme is involved in metabolism of TVA to 9/11CLA after consumption of this compound. Many studies have confirmed that a diet high in TVA results in accumulation of 9/11CLA in fat in serum and tissues of the body. Accordingly, although TVA is a *trans* FA and should be considered to have adverse effects on human health, consumption of this FA is in fact very beneficial due to its metabolic conversion to CLA.

Factors that influence CLA content in cow's milk are diet (type of pasture and quantity consumed, dietary restrictions, oil supplements, amount and type of ration), the production system (animals free in fields, pens, etc.), race and age, the season milk is collected which may involve restrictions on accessibility of pastures rich in polyunsaturated fatty acids. A number of studies have indicated that depending on availability of pastures (which vary with season) and fat supplementation rations (primarily rapeseed or its oil and soybean), the 9/11CLA content in milk can be increased significantly, e.g., by 50% [25]. Consequently, we could expect significant variation in 9/11CLA content in milk from cows, sheep and goats in different countries.

The lipid composition of the cheeses depends on the composition of milk used. Unlike milk or butter, cheese has the advantage of not being perishable in the short term and thus readily available for characterization. For these reasons, fat extracted from cheese was investigated as a convenient way to study milk fat products. FA composition was analyzed by gas chromatography (GC) and TAG composition by high performance liquid chromatography (HPLC) of fatty material extracted from cheeses derived from milk of cow, goat and sheep. Analyses were done with cheeses sold in Uruguay. Our study is one of the first descriptions of FA and TAG content and composition for comparison of dairy products from cow, sheep and goat produced and sold in Uruguay.

2. Materials and Methods

Cow, goat and sheep cheeses were obtained from commercial food stores in Montevideo, Uruguay. All cheeses were produced in Uruguay except samples S3 (sheep) and G4 (goat) that were imported from Spain.

Cow milk cheeses termed Dambo are semi-hard and elastic in consistency. They are not granular fatty cheeses, are uniformly yellowish-white without cracks and have a smooth peel. The flavor is mildly lactic and only slightly salty with little emphasis. Fat was extracted from cheeses using the method of Röse-Gottlieb AOAC 905.02. FA methyl esters were prepared according to IUPAC 2.301 protocol and analyzed by GC using Shimadzu model 14B equipped with a SP 2560 (Supelco) capillary column and a flame ionization detector (FID). The temperature program used was the following: initial temperature 90 °C for 2 min, then increasing to 175 °C at 20 °C/min and maintained for 35 min, then increasing to 240 °C at 15 °C/min and maintained for 25 min. TAG composition was determined by HPLC using Shimadzu model 20A equipped with two columns, Supelcosil™ C-18 (25 cm × 4.6 mm, 5 μm), and an ELSD-LT II detector. The mobile phase (acetone/acetonitrile/chloroform) was run with parameters of Nájera et al. [26]. Molecular characterization of TAGs was done according to Wada et al. [27] in which the parameter (*PN*) partition number is defined: $PN = NC - 2 \times ND$, where *NC* is the number of total carbon atoms of the TAG and *ND* is in number of double bonds in FAs of the TAG molecule. TAGs elute from a reverse phase column in which those with higher PN values are retained and elute later. Elution of TAGs from these columns is in increasing order of PN. Samples were analyzed in duplicate, and peak identification was accomplished through the analysis of authentic standards.

3. Results and Discussion

3.1 Fatty Acids Composition

Fig. 1 shows short-chain FA content (4:0 + 6:0 + 8:0 + 10:0) of cheese fat derived from milk of sheep (S), goat (G) and cow (C) from Uruguay (except samples which correspond to two Spanish cheeses). Cheeses

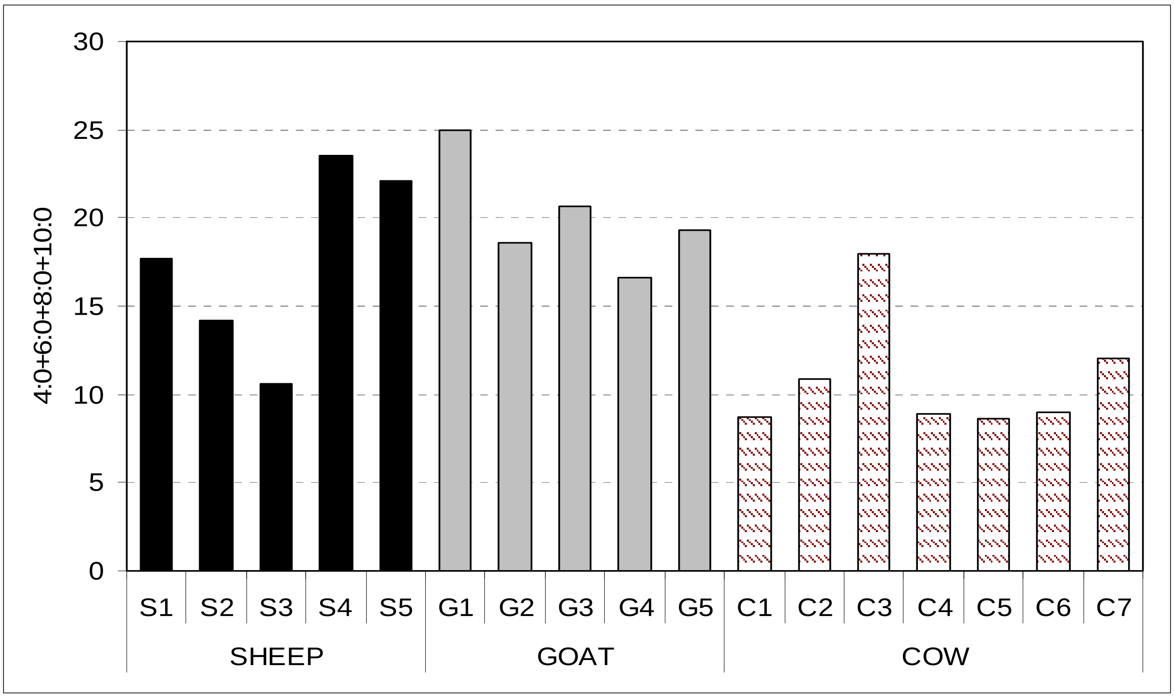

Fig. 1 Short FA content (4:0 + 6:0 + 8:0 + 10:0) in fat of cheeses made from milk of sheep (S), goat (G) and cow (C) from Uruguay (except for S3 and G4 samples that are of Spanish origin).

derived from cow milk have a lower content of these FAs, consistent with the much milder-taste of these cheeses.

Fig. 2 shows variation in content over a one year period of TVA, the remaining *trans* FAs (except CLA) and grams of CLA/100 g Dambo cheese produced by a Uruguayan company. In samples analyzed, the average percentage fat was near 30% with the following composition: saturated FAs, 60%-70%; monounsaturated FAs, 20%-25%; polyunsaturated FAs, 2.0%-3.0%; TVA, 2.9%-4.4%; other *trans* FAs (excluding CLA), 3.5%-6.7% and CLA, 1.0%-1.5%.

As noted above, TVA, despite possessing the *trans* configuration, is very beneficial for human health due to its conversion to 9/11CLA in the cell. The TVA content had an average value higher than 3%, while other *trans* FAs were 1.5% and CLA were found with an average greater than 1.3%.

Variation in 9/11CLA content and that of unconjugated *trans* isomers were similar with respect to month of production in which levels of both either increased or decreased together over a given period of time. This behavior is consistent with the known precursor product relationship for these FA metabolites in rumen hydrogenation and mammary gland dehydrogenation. In addition, Fig. 2 shows seasonal variation with the highest levels of compounds in March and to a lesser extent from June to October. These fluctuations did not affect the consistently high amounts of CLA and TVA in cheeses throughout the year, which in perspective gives them higher nutritional/health value.

Fig. 3 shows TVA content and other *trans* FAs (excluding CLA) of cheeses from milk of sheep, goat and cow (Dambo) from different Uruguayan producers.

The average values for content of *trans* FAs (including TVA but excluding CLA) in cheese fat derived from milk of sheep was 5.2%, from milk of cow, 4.4% with the lowest values for cheese derived from milk of goat, 3.4%. In samples analyzed for Fig. 3, the TVA content was the FA found in the highest amounts indicating health benefit. The average content of TVA for these samples was 3.3% in the fat of sheep cheese, 3.1% for that of cow and 1.8% for goat.

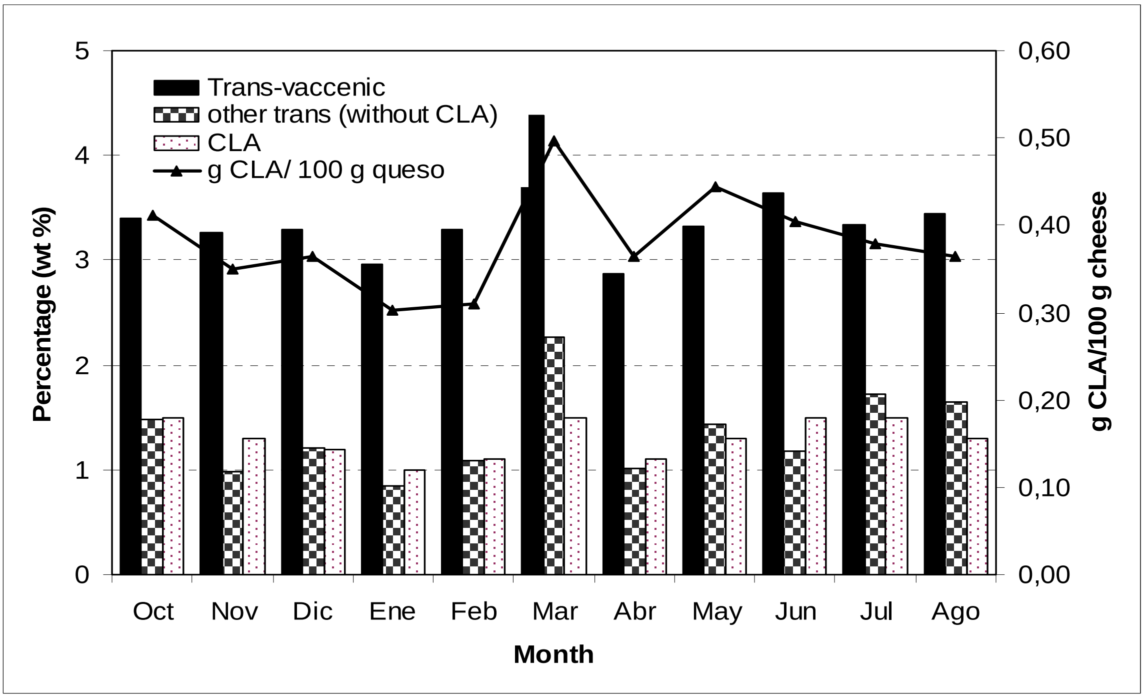

Fig. 2 Variation in content of TVA, CLA and other *trans* FAs (excluding CLA) in Dambo cheeses over one year.

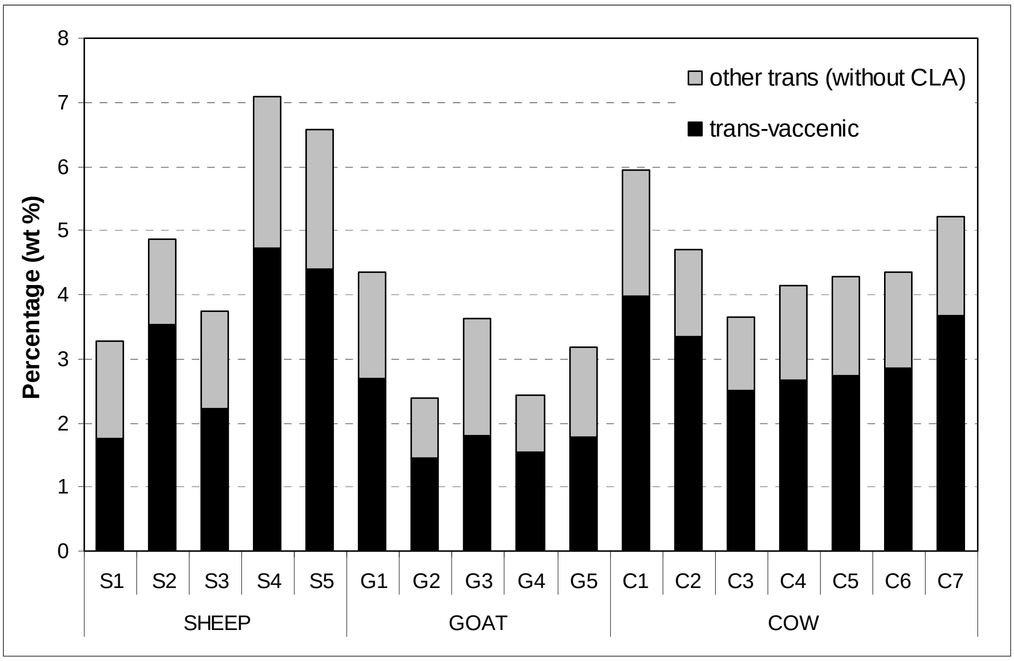

Fig. 3 Content of TVA and other *trans* FAs (excluding CLA) in fat from cheeses derived from milk of sheep (S), goat (G) and cow (C).

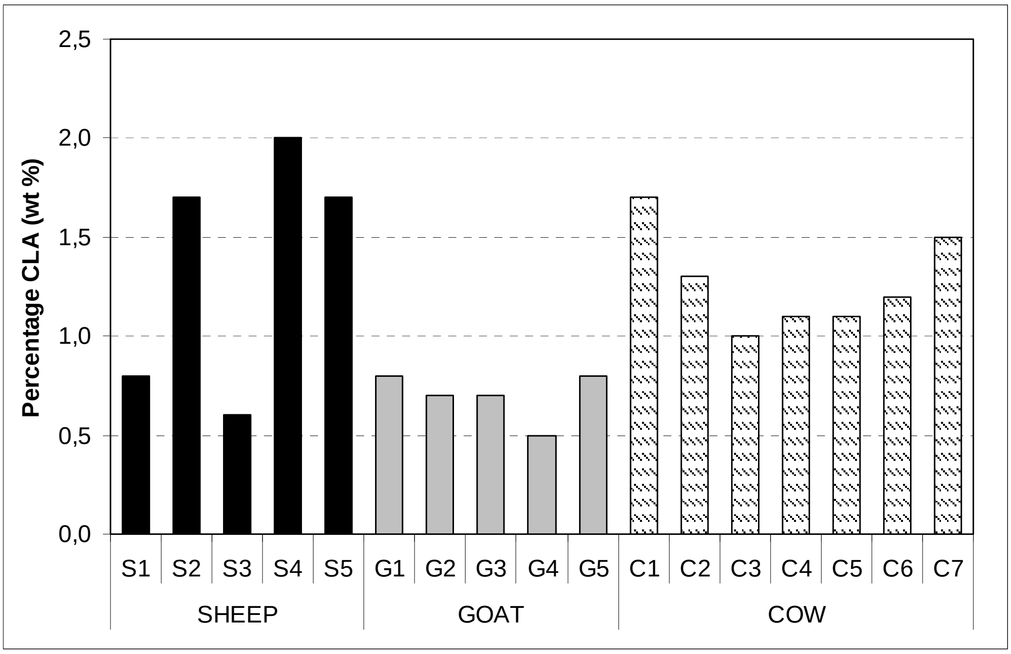

Fig. 4 CLA content in fat from cheeses derived from milk of sheep (S), goat (G) and cow (C).

Fig. 4 shows CLA content in fat from the different types of cheeses (sheep, goat and cow). Goat cheese fat had the lowest average CLA values: 1.4% for sheep cheese, 1.3% for cow and 0.7% for goat.

The relative content of so-called "good" *trans* FAs (CLA + TVA) was higher than that of the remaining *trans* FAs (provisionally, "bad" *trans* FAs). The nutritional or health value of the Uruguayan cheeses (from sheep and cow) from this perspective could thus be considered positive as shown in Fig. 5. The average values for (CLA + TVA) in cheese fat by origin were: 4.7% from sheep, 4.4% from cow and 2.5% from goat.

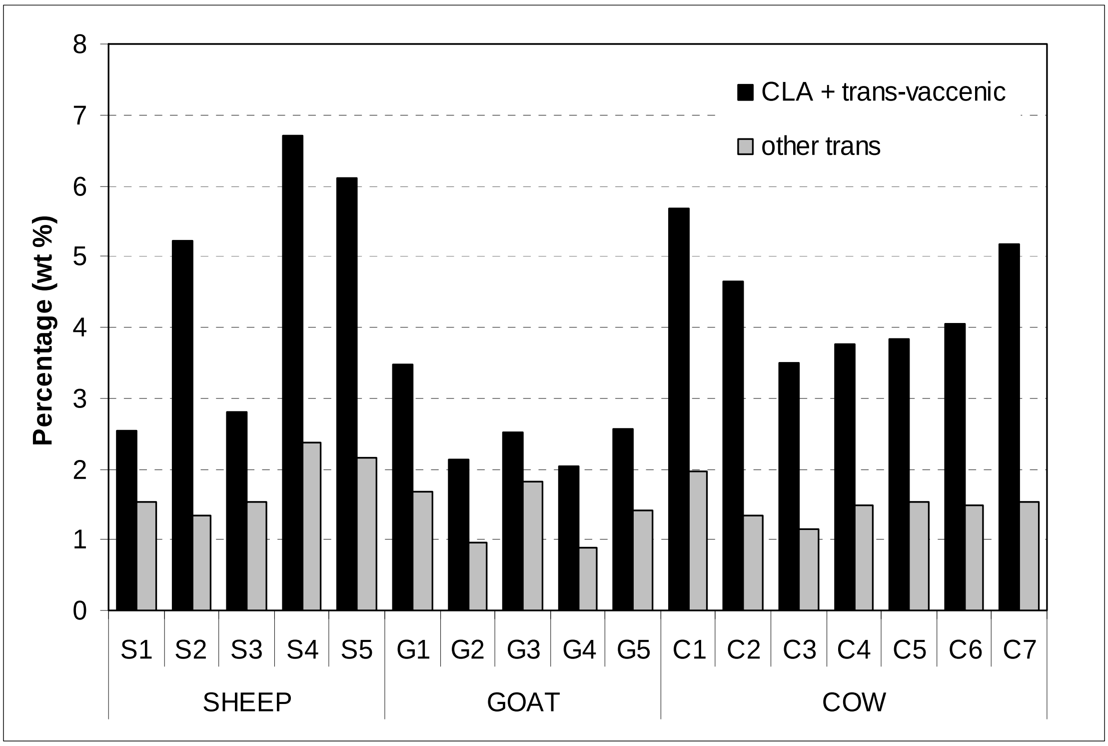

Fig. 5 Content of (TVA + CLA) and other *trans* FAs in fat from cheeses derived from milk of sheep (S), goat (G) and cow (C).

The average values for content of the remaining *trans* FAs were: 1.8%, 1.2% and 1.5%, respectively.

The two cheeses of Spanish origin, samples S3 and G4, had relatively low amounts of CLA and TVA, compared with the most of the other samples of Uruguayan origin (Figs. 3 and 4). The values obtained for S3 cheese were: 0.6% and 2.2% for CLA and TVA, respectively, and for G4 cheese: 0.5% and 1.5% for CLA and TVA, respectively. Previous studies on TVA and CLA content in cheese fat were comparable with our findings except that the levels of these FAs were generally lower. According to Ceballos et al. [10], fat of cow milk they analyzed contained 0.45% CLA and 1.63% TVA (average value for 30 samples), and goat milk fat contained 0.68% CLA and 0.37% TVA (average value for 30 samples). Prandini et al. [7] determined levels of CLA in four Italian cow's milk cheeses: Alpine (0.48%), Emmental (0.77%), Fontina Valdostana (0.81%) and Grana Parmigiano (0.38%). The same investigators found CLA levels in fat of Italian goat's milk cheese: Goat (0.43%) and from sheep, Pecorino (0.78%).

Table 1 shows the average TVA and CLA fat content of commercially available cow, sheep and goat cheese [13].

Zlatanos and Laskaridis [28] determined CLA levels in fat from traditional Greek sheep's cheese: feta (0.72%), Graviera (0.75%) and Manouri (0.54%).

Table 2 shows CLA and TVA content in fat of different commercial cheeses, according to Domagala [12].

One emerging perspective from our study, the others mentioned and others not cited is that, in general, the content of TVA and CLA in fat of milk (and cheese) from cow, sheep and goat in Uruguay is relatively higher than amounts found in milk produced in other countries in which this has been examined. By extension, the higher levels of these potentially beneficial *trans*-FAs in a variety of dairy products from Uruguay may be associated with health or nutritional added value for these products.

Table 1 Content of TVA and CLA in fat from cheeses derived from cow, sheep and goat [13].

Average values	TVA (%)	CLA (%)
Cow cheeses (15 samples)	0.95	0.57
Sheep cheeses (18 samples)	1.63	0.99
Goat cheeses (19 samples)	0.89	0.69

Table 2 TVA and CLA content in fat of commercial cheeses [12].

Cheeses	TVA (%)	CLA (%)
Emmental 1	2.77	0.74
Emmental 2	3.43	1.26
Radamer	2.03	0.71
Mazdamer	2.91	0.98
Dziurawiec	2.50	0.90
Gouda	3.08	1.20
Salami	3.24	1.18
Parmesano	1.36	0.44
Camembert	2.64	0.95
Rokpol	3.69	1.31

3.2 Triglycerides Composition

Fig. 6 summarizes reverse-phase HPLC elution profiles of TAGs in cheese fat derived from Uruguayan sheep, goat and cow milk. There are clearly different elution profiles reflecting different TAG compositions (as different values of TAGs with a given PN) in the three cheese types. The results show the extent of the overall differences in the TAG composition of the three types of cheese.

In general, there were minimal differences comparing cheeses from goat and sheep by this analysis, whereas, a distinct PN profile distribution was evident for TAGs in cow cheese. In general, goat and sheep cheeses have less variation and greater uniformity in TAG distribution for PN between 34 and 50 (having a range in value between 4.4%-16.8%). The PN distribution between 34 and 50 for elution of TAGs in cow cheeses in contrast had three distinct areas. The first area included PN 34 and 36, with values of 7.9% and 18.0%, respectively. The second area was between PN 38 and 44 with values between 3.6%-7.9% and the third area including PN = 46, 48 and 50 where the composition varied between 8.4% and 23.1%. PN values between 28 and 32 for goat and sheep cheeses had a greater proportion of TAGs than cheese for cow in which, e.g., TAG composition for PN = 28 was less than 0.1%. Goat and sheep cheeses at PN = 28 were 0.5% and 0.8%, respectively. For PN = 52, the three cheeses had similar values less than 1.0%.

PN depends on the number of carbons and double bonds present in the TAG. As PN increases, the probability that TAGs are composed of increasingly saturated FAs also increases (mainly as mono unsaturated TAGs) with 14:0, 16:0 and 18:0 as the main saturated FAs. This accounts for why TAG composition values was high for the different cheeses between PN = 40-50. The highest values for goat and sheep cheeses were at PN = 42 and for cow cheeses, PN = 48. The reason why the PN maximum is lower for goat and sheep cheeses might be related to the greater proportion of short chain FAs (4:0 + 6:0 + 8:0 + 10:0) in these cheeses compared with cow cheeses as shown in Fig. 1. This is particularly evident for differences in the values for 10:0, with average values of 11.5% and 8.6% for sheep and goat cheeses respectively and only 2.8% for cow cheeses.

Considering the four FAs present in greater proportion among the three types of cheese (10:0, 14:0, 16:0 and 18:1), the TAGs 10:0/14:0/16:0 and 10:0/16:0/18:1 with PN = 40 and 42, respectively are in low amounts in cow's cheese and will major in goat and sheep cheeses, this would explain the results obtained in this study. Also, the highest PN value for cow cheeses was 48, reflecting the presence of longer chain FAs in cow milk fat TAGs. FAs 14:0, 16:0 and 18:0 were present at highest levels and most were monounsaturated. As such, TAGs 16:0/18:1/18:1, 14:0/18:0/18:1 and 16:0/16:0/18:1 in which PN = 48 were the most abundant. According to Nájera et al. [26], predicted TAGs with PN = 42 are 10:0/16:0/12:0, 16:0/14:0/16:0, 8:0/16:0/18:0, 14:0/14:0/14:0, 6:0/18:0/18:0 and with PN = 48 predicted TAGs are, 16:0/16:0/18:1 and 14:0/18:0/18:1. Other studies predicted TAGs based on observed PN values. Weber et al. [29] found the majority of cow's milk fatty

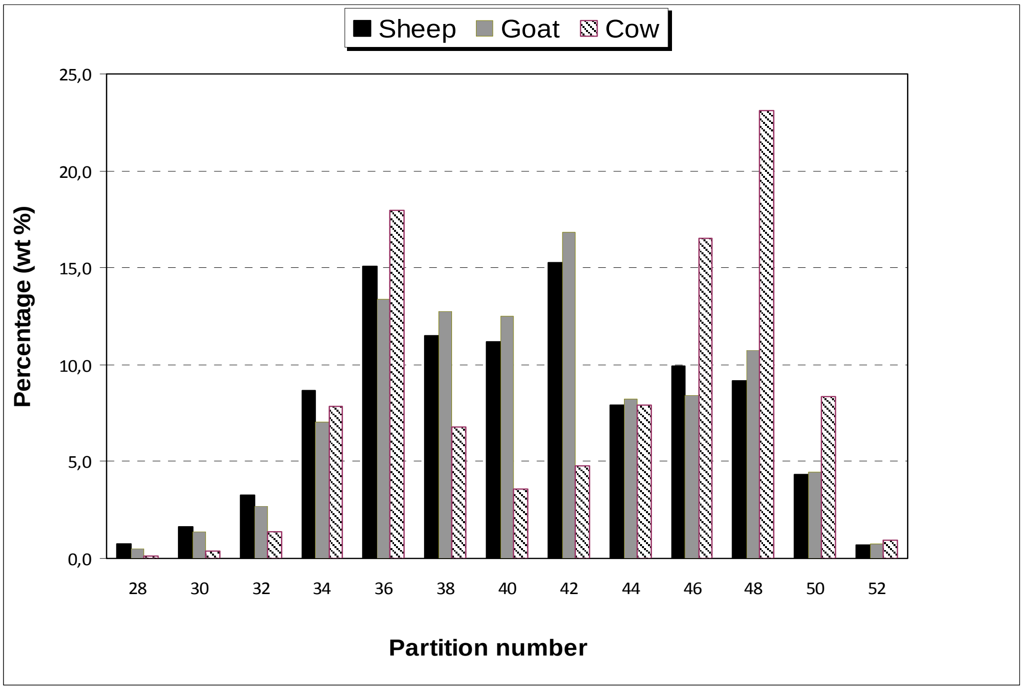

Fig. 6 Average composition of TAGs (%) depending on the partition number (PN) for cow, goat and sheep cheeses.

material TAGs to be PN = 36 (4:0/16:0/4:0, 16:0/14:0/18:0, 4:0/16:0/18:1 and 10:0/14:0/16:0) and PN = 48 (16:0/16:0/18:1, 14:0/18:0/18:1 and 16:0/18:1/18:1). Barron [30], however, identified the majority of fractions for TAGs with PN = 46 (14:0/18:1/18:1), PN = 44 (14:0/18:1/12:0 and 18:2/16:0/16:0) and PN = 34 (4:0/14:0/18:1 and 4:0/14:0/16:0).

In summary, the FA composition of goat and sheep cheeses consists of a larger number of short-chain FAs (4:0, 6:0, 8:0 and 10:0), such that it can be expected that TAG composition can be described as having numerically lower PN values. This accounts for why these cheeses have TAG compositions with PN values for TAGs between 28 and 32 that are higher than those of cow cheeses.

4. Conclusions

Despite seasonal variations, Uruguayan cow's milk cheeses analyzed had consistently higher levels of both CLA and TVA compared with those of other national origins. The relatively higher levels of these compounds would be consistent with regional milk-related products as having health or nutritional added value compared with related products of other national origins. A high content of "good" *trans* FAs (TVA + CLA) has also been found in other cow's milk-based products (e.g., butter). This is a very positive attribute for commercialization of these products, which differentiates them from related products from other countries.

Goat and sheep Uruguayans cheeses are also richer in "good" *trans* fatty acids (TVA + CLA) than the corresponding cheeses from other countries. In general, Uruguayan sheep and cow cheeses are a little more beneficial to health, from the point of view of the amount of TVA and CLA which possess, compared to the Uruguayan goat cheeses.

In close relation with that, the TAG composition of the fatty material of the cheeses analyzed is in accordance with the composition of fatty acids; cow cheeses presented TAGs with greater percentages of PN than goat and sheep cheeses, reaching the maximum at 48 and 42, respectively, reflecting the higher proportion of FA with short chain in cheeses from goat and sheep compared with what was found in cow cheeses.

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